Interagency Agreement: U.S. Food & Drug Administration National Center for Toxicological Research

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Background and Rationale

An Interagency Agreement (IAG) was established in 1992 between the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences (NIEHS) and the National Center for Toxicological Research (NCTR) of the U.S. Food & Drug Administration (FDA). The purpose of the IAG is to provide funding to support toxicology studies on FDA-regulated chemicals that have been nominated to the NTP. These studies are designed to provide the FDA and other regulatory agencies with hazard identification and dose-response data to support risk assessment and risk management decisions that could affect public health.

Goals and Objectives

The primary goals of the IAG are to: (1) Support the design and execution of toxicological studies that are consistent with the goals and needs of the FDA and NTP; (2) Provide oversight to ensure that the studies are conducted in the most rigorous scientific manner; (3) Ensure the data resulting from the studies are available to agencies to enable science-based, safety assessment and risk management decisions.

The goals of the IAG are met through the following objectives for each of the studies that are conducted under the IAG: (1) Implement validated toxicology studies (such as the NTP guideline studies); (2) When appropriate, conduct studies with innovative designs to meet the needs of the FDA and/or NTP; (3) Incorporate appropriate mechanistic studies to assist in study interpretation, identification of potential translational biomarkers, and safety and risk assessments; (4) Provide the study data to the FDA regulatory and scientific community in a timely manner to provide the basis for risk assessments.

Organization

Chemicals or substances that are studied under the IAG have been previously reviewed by the NTP Interagency Chemical Coordination and Evaluation Committee (ICCEC), NTP Board of Scientific Counselors (BSC), and NTP Executive Committee (EC). Nominations of chemicals or substances to the NTP for toxicological testing may come from the FDA, other government agencies, professional organizations, health care professionals, or private individuals. Regardless of the source of the nomination, chemicals or substances that are regulated by the FDA may be selected for study under the IAG.

The IAG is organized and managed by the NTP Project Officer, with an FDA Project Officer at NCTR for on-site management. The conduct and progress of the studies under the IAG are monitored by the IAG Toxicology Study Selection and Review Committee (TSSRC). The TSSRC is composed of the NCTR Project Officer (TSSRC Chair), NTP Project Officer, NCTR Director, project leaders from NCTR, scientists from

NTP and NIEHS, scientists from each of the FDA product centers (CBER, CDER, CDRH, CFSAN, and CVM), and invited subject matter experts from government, academia, or industry.

The TSSRC meets twice yearly with the following objectives/tasks: (1) Review the toxicology research plans developed by the NCTR principal investigators for specific chemicals or substances in response to the nominations; (2) Recommend modifications to the research project plans or alternative studies that will enhance the regulatory utility of the studies that will be conducted under the IAG, taking into consideration that multiple toxicological endpoints may be required for risk assessments (*e.g.* carcinogenicity, reproductive/developmental toxicity, neurotoxicity, behavior alterations, immunotoxicity, mechanistic studies); (3) Consider and recommend alternative test systems that may meet the data needs of the regulatory agencies, such as the use of genetically modified mice or *in vitro* test systems; (4) Review the study protocols for appropriateness of the doses and study design and dose-selection to maximize the information for hazard identification and dose-response determination; (5) Monitor the progress of each project through reviewing the preliminary and final data; (6) Provide input into alternative endpoints or studies that may strengthen risk assessment and regulatory policy decisions that will affect public health.

Project Outcomes

The IAG to date has supported projects on 37 different chemicals or test articles that were of regulatory or scientific interest to the FDA and NTP. The projects are briefly outlined below, and have resulted in 12 published NTP Technical Reports (TR 496, 502, 503, 508, 510, 524, 527, 539, 545, 547, 548, and 553) with 3 technical reports being reviewed in the upcoming NTP BSC Technical Report Review Subcommittee (TRRS) meeting (winter 2009). These studies have also resulted in 162 scientific publications.

The program areas are briefly discussed below. The Technical Reports, publications, and accompanying program booklet can be consulted for more information.

• Endocrine Active Agents Program

There are many chemicals or compounds with endocrine activity, including estrogenic or androgenic activities. The NCTR and NTP established a program to evaluate endocrine active agents.

The **Multigeneration Studies Program** was designed to determine the reproductive and lifetime risk of estrogenic compounds. Genistein, found in soy products, and ethinyl estradiol, a component in many birth control medications were examined in both a five-generation reproductive study and a two-year bioassay. Effects were detected in exposed animals from these studies. The five-generation reproductive study on nonylphenol, an intermediate in the production of surfactants and other industrial products, revealed only limited kidney toxicity. Limited studies were conducted with vinclozolin, and methoxychlor. These studies have been completed and published.

Bisphenol A is another compound with reported estrogenic activity. It is included in polycarbonate plastics as a polymerizing agent, and has been reported to leach from the plastics into liquids. The FDA and NTP requested that pharmacokinetic studies be conducted on bisphenol A in rats and non-human primates, and the data be used to

establish a physiologically based pharmacokinetic (PBPK) model to understand the distribution of bisphenol A into human tissues. In addition, the IAG is supporting subchronic studies following perinatal through 90 day exposure of rats to bisphenol A. These studies are on-going at this time, and the goal is to develop an understanding of the toxicity of bisphenol A to humans at low doses.

• Dietary Supplements Program

The IAG has supported studies on dietary supplements. The FDA regulates dietary supplements under the Dietary Supplement Health and Education Act, which states that the dietary supplement manufacturer is responsible for ensuring that dietary supplements are safe before being marketed. Although the manufacturers are responsible for registration, safety, and labeling accuracy, a paucity of toxicology data on available dietary supplements has resulted in the nomination of many dietary supplements to the NTP.

Riddelliine is a pyrrolizidine alkaloid that is present in many botanical plants in the US. The NTP conducted a 2-year chronic bioassay on riddelliine, and researchers at NCTR supported the studies by the identification and quantification of the DNA adduct resulting from riddelliine metabolism. Data from these studies were used to support the listing of riddelliine in the Report on Carcinogens.

Aloe vera is included in many products for topical application and oral consumption. The use of the product dictates the plant fraction used. Studies were conducted under the IAG on the chronic toxicity of whole leaf aloe vera juice extract in rats and mice. The results of this study will be presented at the winter 2009 NTP BSC TRRS meeting.

Usnic acid is a secondary metabolite of lichen in the *Usnea* genus. Usnic acid has been used as a traditional medicine, and is also present in some weight loss dietary supplements and commercial products. The FDA has received reports of hepatic toxicity in humans following consumption of usnic acid. Subchronic toxicity studies are being conducted to establish a dose-response and mechanism of action of usnic acid and *Usnea* lichen in rats and mice. These studies are on-going.

Bitter Orange, or ***Citrus aurantium***, extracts have been used as a substitute for ephedra following the FDA ban on ephedra-containing products. These extracts contain sympathomimetic compounds including synephrine. Studies have been completed on the developmental toxicity of extracts. Physiological and cardiovascular effects of bitter orange in resting and exercising rats are on-going. All of the studies also include a testing arm with caffeine, which is consistently used in conjunction with bitter orange extracts.

• Food Contaminants Program

The FDA is responsible for assuring the safety of the US food supply. This responsibility extends over natural ingredients in foods or contamination by bacteria or fungi, and through anthropomorphic activities including cooking or the addition of flavors, preservatives, or colors. In addition, chemicals can migrate into foods and other products following contact with non-food materials (*e.g.* packaging) or may be illegally introduced to create an adulterated product. The FDA has requested toxicology studies

on chemicals or substances to reduce the uncertainty of the risk of some of these materials.

Fumonisin B1 is a fungal metabolite produced by fungi of the *Fusarium* genus. *Fusarium* fungi contaminate corn and other food as the result of plant stress. The studies conducted under the IAG established that fumonisin B1 is a renal carcinogen in rats and a hepatocarcinogen in mice. The dose-response in the IAG studies was used to establish maximal tolerated levels in foods worldwide.

Urethane is a by-product of fermentation and occurs at significant concentrations in wines, brandy, and other liquors. The FDA did not have reliable dose-response studies on the carcinogenesis of dietary urethane, and was concerned that ethanol could potentiate urethane carcinogenesis. Chronic standard two-year bioassay indicated that ethanol had a weak/mixed effect on the carcinogenesis of urethane. Urethane levels were reduced in distilled spirits subsequent to these studies.

Acrylamide is produced by some food processing procedures, and occurs in some food at high levels. Although some information existed on the carcinogenicity and neurotoxicity of acrylamide in rodents, the FDA determined that the data were inadequate for a human risk assessment of acrylamide. Neurotoxicity studies in rats, and rodent chronic bioassays of acrylamide, and its metabolite glycidamide, have been conducted under the IAG, and the results will be reported early in 2010.

Malachite Green is an antifungal triphenyl methane drug. It is illegal to use this compound in aquaculture in fish hatcheries in the US; however, its use is reported worldwide. The two-year bioassay conducted under the IAG established a dose-response of rodent carcinogenesis to malachite green, and the data was used by the FDA to reinforce its position that malachite green should not be present in human food. Other countries used the data to support dose-hazard assessments.

Furan is a volatile carcinogenic compound that is formed when polyunsaturated fatty acids, carbohydrates, and vitamin C are heated during cooking or processing. The NTP had conducted a 2-year bioassay with furan in the late 1980's, and established that furan induced hepatocellular carcinomas, cholangiocarcinomas, and leukemia in rodents; however, the very high tumor response in the lowest dose groups created a considerable uncertainty in the risk assessment. A two-year bioassay, mechanistic and pharmacokinetic studies are being conducted to provide data for a risk assessment of dietary furan.

Melamine, alone or in combination with **Cyanuric Acid**, has induced renal failure in cats and dogs as the result of contamination of animal feed, and more recently has induced renal failure in infants following consumption of contaminated milk products in China. The FDA has requested short term and mechanistic studies be conducted in rats and pigs to provide additional data to help establish a no-adverse effect level and to support a risk assessment model of melamine. These studies are on-going at this time.

• AIDS Therapeutics Program

Human acquired immune deficiency syndrome (AIDS) is caused by a retroviral infection by the human immunodeficiency virus (HIV). Antiviral therapy is used to control the propagation of the virus and spread of the disease in infected individuals, and is used to

treat pregnant mothers and newborns (prophylactically) to prevent mother-to-offspring infection of the newborn. Although this has reduced the HIV infection rate in newborns, the long-term consequences of administration of DNA base analogues and nuclease inhibitors have not been established. The IAG has supported studies on the effects of AIDS therapeutic combinations in rodent bioassays.

Mice have been dosed transplacentally with the combination of Zidovudine, Nevirapine, Lamivudine, Nelfinavir, and Efavirnez and the carcinogenesis outcome was monitored for 2-years. The results of this study will be reviewed at the NTP BSC TRRS in winter 2009.

In a second study, mice were dosed transplacentally and during the first eight days of life with the combination of Zidovudine, Nevirapine, Lamivudine, Nelfinavir, and Efavirnez and the carcinogenenic outcome is being monitored for two years. The results of this study will be reviewed in 2010.

In a third study, genetically modified B_6C3F1 mice were dosed with the combination of Zidovudine, Nevirapine, and Lamivudine transplacentally and during the first 28 days of life, and the carcinogenesis outcome was monitored for nine months. The results of this study will be reviewed at the NTP BSC TRRS in winter 2009.

• Pediatric Program

Many toxicological studies that support drug safety evaluations for use in adults are not required to evaluate the safety of the drug in a neonate or prepubescent animal model. Since many drugs are clinically used beyond the initial marketing intent, several drugs are being used in pediatric patients where no clear evaluation of age-dependent toxicity has been conducted. Several studies have been requested by the FDA to provide data to support risk assessment of pediatric exposure.

Chloral Hydrate is a hypnotic used in pediatric medicine, and was genotoxic in several *in vitro* assays. Mice were treated as neonates with chloral hydrate, and the studies concluded that there was no clear evidence of genotoxicity or carcinogenicity. In a separate study, male mice were dosed for two years with dietary restriction, and the results showed an induction of liver tumors indicative of peroxisome proliferation. These results led the FDA to conclude that risk of pediatric use of chloral hydrate was minimal and did not require medical practice changes.

Di(2-ethylhexyl)phthalate (DEHP) is a plasticizer used in medical devices that have contact with blood, serum, dextrose, and saline, and is found in intravenous tubing and dialysis equipment. It has been known that DEHP migrates from the medical devices into the fluids and can result in human exposure. The FDA became concerned regarding the magnitude of DEHP dose that could be received by neonates in intensive care facilities. Studies are on-going to determine the pharmacokinetics of DEHP following administration to neonate rodents and non-human primates.

Ketamine is a representative of a group of anesthetics which have been reported to cause widespread and dose-dependent apoptotic neurodegeneration in rodents after birth. The FDA requested studies be conducted to confirm and extend the observations of neuronal toxicity following juvenile administration. Studies are on-going to establish

the apoptotic potential of ketamine in primary cultures of rodent and non-human primate neural cells and *in vivo* in young non-human primates.

• Electromagnetic Radiation Program

The U.S. public is exposed to many different wavelengths and intensities of electromagnetic radiation. In some of these exposures, the nature and intensity of the exposure has resulted in concern regarding public safety. Two specific programs are underway to evaluate human risk to some electromagnetic radiation sources.

Cellular Telephone Radiation

The use of cellular telephones has dramatically increased over the past decade to be a primary form of communication among all age groups. Some epidemiological studies suggested an association of cellular telephone use with brain cancer. Since it regulates electromagnetic radiation producing devices, the FDA nominated **cellular telephone radiation** to the NTP for toxicity and carcinogenicity studies. The NTP is currently conducting range-finding and long-term studies on cellular telephone radiation studies in rodents. The FDA requested, and the IAG will support *in vitro* studies on the effects of cellular telephone radiation frequencies on neural cells in culture, and will support the histopathological analysis of blood brain barrier function in brain tissues from the *in vivo* studies.

Phototoxicity with Simulated Solar Light and UV Light Sources

In the late 1990's the FDA and NTP recognized the need for a facility to conduct studies on the toxicity of chemicals and substances in the presence of natural sunlight. The Center for Phototoxicology was established in 1999, and has developed into one of only two facilities in the world capable of conducting rodent toxicity or carcinogenicity studies with simulated solar light.

Alpha and Beta Hydroxy Acids are included in many cosmetic products as keratolytic agents to correct age and sunlight induced wrinkles. The application of cosmetics containing these acids results in restructuring of the skin and proliferation of the epidermis basal cells. Studies were conducted under the IAG to determine the photocarcinogenesis of topically applied **alpha hydroxy acid (glycolic acid)** and **beta hydroxy acid (salicylic acid)** following 40 weeks of dosing with and without simulated sunlight on hairless mice. The conclusions from the studies indicated that glycolic acid did not increase the carcinogenesis of simulated solar light, and that salicylic acid decreased the induction of skin cancer by simulated solar light.

Components of the plant **aloe vera** are included in many products that are topically applied or come in contact with human skin. Publications in the scientific literature suggested that some components of aloe vera could be phototoxic or photocarcinogenic. Studies were conducted under the IAG and demonstrated that the topical application of creams containing aloe gel, aloe whole leaf, decolorized aloe whole leaf, and aloe-emodin had a marginal effect on the carcinogenesis of simulated solar light.

Retinyl Palmitate is an ester of vitamin A and is included in many cosmetic products. Topical applications of retinoic acids have been shown to enhance the photocarcinogenesis of light sources containing ultraviolet light. The FDA requested

studies to examine the effect of topically applied retinyl palmitate on simulated solar light induced skin cancer. The results of these studies will be presented at the winter 2009 NTP BSC TRRS.

Permanent Makeup Pigments are a mixture of inorganic and organic pigments, thickening compounds, and botanicals in an alcohol-water solvent. Starting in 2003, the FDA received reports that women responded adversely to products from a manufacturer of permanent makeup inks. The product lines were recalled, and the FDA requested that the NTP assist in determining if components of the inks could induce an immune response in animal models. These studies are ongoing and have demonstrated that the permanent makeup inks elicit a positive response in a modification of the local lymph node assay.

Lemon and lime oil are present in many products regulated by the FDA. These oils contain photoactive **furocoumarins**, and the chemical and concentration are dependent on the plant species, growth conditions, and processing. The FDA requested studies be conducted to determine if furocoumarins such as oxypeucedanin from lime oil could be photoactivated. The completed studies demonstrated that oxypeucedanin, coumarins, and furocoumarins present in lemon and lime oil were photoactivated *in vitro* to form DNA adducts.

• Nanoscale Materials Program

Nanoscale materials are defined as substances specifically synthesized with at least one dimension between 1 and 100 nm. Some materials in this size range have unique properties that are not present in larger size crystals or particles of the same material. The unique physicochemical properties that result in the unique applications of nanoscale materials, may present unique toxicity when in contact with biological systems. The NTP has established a Nanotechnology Safety Initiative in response to this need to understand nanomaterial safety. Similarly, the FDA has recognized the need for understanding the toxicological potential of nanoscale materials.

Nanoscale **Titanium Dioxide and Zinc Oxide** are included in many products including cosmetics and sunscreens. The IAG supports a program to determine the biodistribution of intradermally administered nanoscale quantum dots and nanoscale titanium dioxide, and also to determine if topical application of these materials results in dermal penetration and biodistribution. Depending on the results of these studies, subchronic or phototoxicity studies may be conducted.

Nanoscale Silver is marketed as a dietary supplement and home remedy for many ailments, and is bacteriostatic and bacteriocidal. The FDA requested the NTP conduct studies to determine the effect of particle size on the bioavailability and toxicity of nanoscale silver. The studies are ongoing at this time.

Nanoscale gold is being used as a nanoscale platform for the construction of drugs, devices, and other therapeutics. There is a paucity of information regarding the effect of nanoscale gold size on the biodistribution and toxicity in animals. The FDA requested the NTP design studies to determine these fundamental aspects of nanoscale gold, and the studies are currently being designed.

Significance and Public Health Impact of IAG

The studies that have been completed under the IAG have resulted in FDA regulatory decisions that will affect public health. Some of the data from the IAG-supported studies have led to increased understanding of the pharmacokinetics, mechanism of action, or dose-response of a chemical or substance. Other data has led to refinement of risk assessment models, while the results of some studies indicated that the chemical or substance was not toxic or carcinogenic to animals and did not pose a risk to humans.